CLAIMS

1. Method for the transformation of plastid genomes of plant species, in particular *Asteraceae* plant species, comprising the steps of:

a) providing a transformation vector carrying a DNA sequence of interest;

b) subjecting a plant material, which comprises plastids, to a transformation treatment in order to allow the plastids to receive the transformation vector;

c) placing the thus treated plant material for a period of time into contact with a culture medium without selection agent;

d) subsequently placing the plant material into contact with a culture medium comprising a selection agent; and

e) refreshing the culture medium comprising a selection agent to allow plant material comprising plastids that have acquired the DNA of interest to grow into transformants.

2. Method as claimed in claim 1, wherein the expression vector comprises:

- an expression cassette which comprises optionally a promoter active in the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA sequence encoding a transcription termination region active in the plant species to be transformed,

- optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest, and

- optionally a DNA sequence encoding a gene of interest inserted into the insertion site of the expression cassette.

3. Method as claimed in claim 2, wherein the vector comprises the promoter, the DNA sequence encoding the gene of interest the one or more selection markers and the set of DNA targeting segments.

4. Method as claimed in any one of the claims 1-3, wherein the transformants carry the DNA of interest in their genome.

5. Method as claimed in any of the claims 1-4, wherein the plastids to be transformed are selected from the group consisting of chloroplasts, amyloplasts, elaioplasts, etioplasts, chromoplasts, leucoplasts and proplastids.

6. Method as claimed in any one of the claims 2-5, wherein the promoter is selected from the group consisting of the chloroplast specific ribosomal RNA operon promoter rrn (16S rRNA), psbA, rbcL, trnV, or rps16.

7. Method as claimed in any one of the claims 2-6, wherein the DNA of interest is a gene encoding a therapeutic or prophylactic (bio)pharmaceutical (poly)peptide, such as an edible vaccine.

8. Method as claimed in any one of the claims 2-6, wherein the DNA of interest is selected from the group consisting of genes encoding herbicide resistance, insect resistance, fungal resistance, bacterial resistance, genes that lead to stress tolerance, for instance to cold, high salt or minerals, genes that improve yield, starch accumulation, fatty acid accumulation, photosynthesis.

9. Method as claimed in any one of the claims 2-8, wherein the terminator is selected from the group consisting of the *psb* A termination sequence, rrn, rbcL, trnV, or rps16.

10. Method as claimed in any one of the claims 1-9, wherein the selection marker is selected from the group

consisting of genes conferring resistance against spectinomycin, streptomycin, kanamycin, hygromycin and chloramphenicol, glyphosate, bialaphos.

11. Method as claimed in any one of the claims 1-9, wherein the selection marker is a visual markers, such as a fluorescent marker like gfp (green fluorescence protein).

12. Method as claimed in claim 11, wherein the steps d) and e) of the transformation method are omitted and the transformants are selected by illuminating the putative transformants with an appropriate light source corresponding to the visual marker and selecting the plant material that shows fluorescence.

13. Method as claimed in any one of the claims 2-12, wherein the DNA segments that allow double homologous recombination of the DNA of interest with the plastid genome of interest have a DNA sequence that is homologous to a part of the plastid genome.

14. Method as claimed in claim 13, wherein the set of DNA segments is selected from the group consisting of the *trn*I(*ori*A)/*trn*A region and the 16S/*trn*V/ORF70B region of the lettuce chloroplast genome.

15. Method as claimed in claim 13, wherein the set of DNA segments is selected from LCV1 A-B and LCV1 C-D, and LCV2 A-B and LCV2 C-D.

16. Method as claimed in any one of the claims 1-15, wherein the transformation treatment is selected from the group consisting of electroporation, particle gun transformation, polyethylene glycol transformation and whiskers technology.

17. Method as claimed in any one of the claims 1-16, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture

medium without selection agent is 1 to 14 days, preferably 3-7 days, more preferably about 6 days.

18. Method as claimed in any one of the claims 1-16, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 14 days, preferably 1-5 days, more preferably about 2 days.

19. Method as claimed in any one of the claims 1-18, wherein the plant material to be treated is selected from the group consisting of plant tissue, separate cells, protoplasts, separate plastids.

20. Method as claimed in any one of the claims 1-19, wherein the culture medium comprising the selection agent is a liquid medium.

21. Method as claimed in any one of the claims 1-20, wherein step c) is performed in the dark.

22. Vector for the transformation of plastid genomes of plant species, in particular *Asteraceae* plant species, which vector comprises:

- an expression cassette which comprises optionally a promoter active in the plastids of the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA sequence encoding a transcription termination region active in the plastids of the plant species to be transformed, and

- optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest.

23. Vector as claimed in claim 22, wherein the vector comprises the promoter, the one or more selection markers and the set of DNA targeting segments.

24. Vector as claimed in claim 22 or 23, which vector comprises:

- an expression cassette which comprises a promoter active in the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a terminator active in the plant species to be transformed, and

- a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest.

25. Vector as claimed in any one of the claims 22-24, further comprising a DNA sequence of interest inserted into the insertion site of the expression cassette.

26. Vector as claimed in any one of the claims 22-25 for use in the method as claimed in any one of the claims 1-20.

27. Vector as claimed in any one of the claims 22-26, wherein the promoter is selected from the group consisting of the chloroplast specific ribosomal RNA operon promoter rrn (16S rRNA), psbA, rbcL, trnV, or rps16.

28. Vector as claimed in any one of the claims 21-26, wherein the DNA of interest is a gene encoding a therapeutic or prophylactic (bio)pharmaceutical (poly)peptide, such as an edible vaccine.

29. Vector as claimed in any one of the claims 22-27, wherein the DNA of interest is selected from the group consisting of genes encoding herbicide resistance, insect resistance, fungal resistance, bacterial resistance, genes

that lead to stress tolerance, for instance to cold, high salt or minerals, genes that improve yield, starch accumulation, fatty acid accumulation, photosynthesis.

30. Vector as claimed in any one of the claims 22-29, wherein the terminator is selected from the group consisting of the *psb* A termination sequence, rrn, rbcL, trnV, or rps16.

31. Vector as claimed in any one of the claims 22-30, wherein the selection marker is selected from the group consisting of genes conferring resistance against spectinomycin, streptomycin, kanamycin, hygromycin and chloramphenicol, glyphosate, bialaphos.

32. Vector as claimed in any one of the claims 22-30, wherein the selection marker is a visual marker, such as fluorescent markers like gfp (green fluorescence protein).

33. Vector as claimed in any one of the claims 22-32, wherein the DNA segments that allow double homologous recombination of the DNA of interest with the plastid genome of interest have a DNA sequence that is homologous to a part of the plastid genome.

34. Vector as claimed in claim 33, wherein the set of DNA segments is selected from the group consisting of the *trn*I(*ori*A)/*trn*A region and the 16S/trnV/ORF70B region of the lettuce chloroplast genome.

35. Vector as claimed in claim 34, wherein the set of DNA segments is selected from LCV1 A-B and LCV1 C-D, and LCV2 A-B and LCV2 C-D.

36. Transplastomic plant or plant part obtainable by the method as claimed in any one of the claims 1-21.

37. Transplastomic plant or plant part as claimed in claim 33 obtained by the method as claimed in any one of the claims 1-21.

38. Transplastomic plant or plant part as claimed in claim 36 or 37, wherein the plant is a lettuce plant.

39. Progeny of a plant or plant part as claimed in any one of the claims 36-38 carrying plastids at least part of which have the gene of interest in their genome.

40. Plant parts as claimed in any one of the claims 36-39, which plant parts are selected from the group consisting of tissues, cells, meristems, calli, protoplasts, plastids, proplastids, plastid DNA.